PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61L 15/40 — A1

(11) International Publication Number: **WO 97/06835**

(43) International Publication Date: 27 February 1997 (27.02.97)

(21) International Application Number: PCT/GB96/01959

(22) International Filing Date: 12 August 1996 (12.08.96)

(30) Priority Data:
9516556.9 12 August 1995 (12.08.95) GB

(71) Applicant *(for all designated States except US)*: SMITH & NEPHEW PLC [GB/GB]; 2 Temple Place, Victoria Embankment, London WC2R 3BP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: RICHARDSON, Mark, Christopher [GB/GB]; Pear Tree Cottage, Main Street, Huby, York YO6 1HS (GB). BLOTT, Patrick, Lewis [GB/GB]; 3 Newland Park Close, York YO1 3HW (GB). MATTHEWS, Jane, Bridget [GB/GB]; 84 South Bank Avenue, York YO2 1DP (GB). DREWERY, George, Robert [GB/GB]; Highfield, 13 Goodmanham Road, Market Weighton, North Humberside YO4 3HT (GB).

(74) Agent: SMITH & NEPHEW GROUP RESEARCH CENTRE; Corporate Patents & Trade Marks Dept., Heslington, York YO1 5DF (GB).

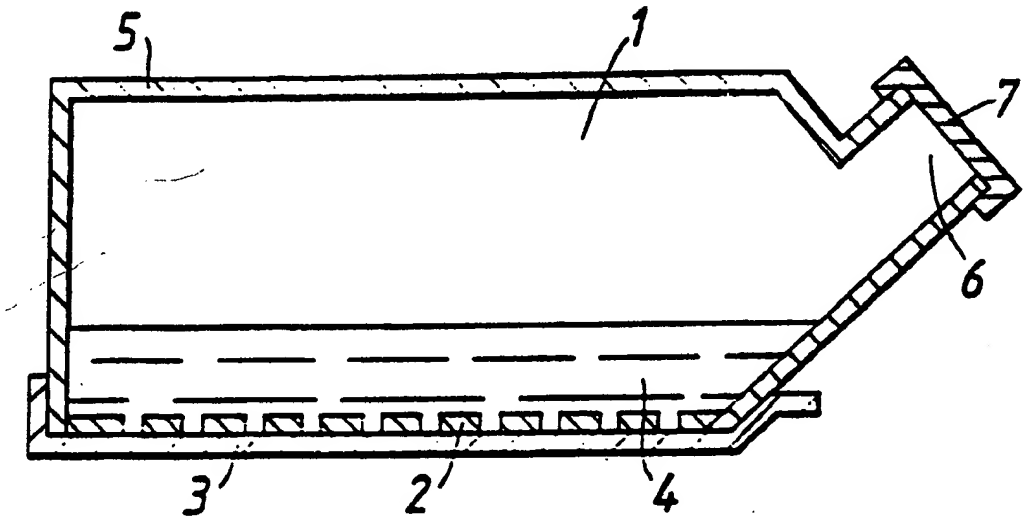
(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CELL CULTURE PRODUCTS



(57) Abstract

A wound dressing comprises a carrier comprising a hydrophilic polymer layer and a layer of cultured mammalian cells anchored to the wound-facing surface of the carrier. A method of making such a dressing is also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

CELL CULTURE PRODUCTS

This invention relates to the culturing of mammalian anchorage dependent cells onto a conformable carrier. More particularly the invention relates to the formation of wound dressings suitable for treating wounds such as burns, skin graft donor sites and ulcers e.g. venous and decubitus ulcers and to systems for use in the preparation of such dressings.

Mammalian cells that are incapable of proliferating in suspended liquid culture but can be made to proliferate on the surface of a carrier are said to be anchorage-dependent.

Epithelial cells, such as keratinocytes, are anchorage dependent. Such cells cultured in the presence of a carrier which is non-inhibitory and non-cytotoxic will multiply in stratified colonies and eventually produce a confluent layer. Cell cultures of this type are used to investigate skin growth and have been used as skin grafts. Various technical papers have been published which describe in vitro techniques for growing skin cells and their subsequent use in the treatment of full-thickness wounds. For example E. Bell et al (J Invest Derm 81; 2s-10s 1983); E. Bell et al (Science 211; 1052-1054 1981); D. Asselineau and M. Pruneiras (Br J Derm 1984 III, Supplement 27, 219-222) and J.F. Burke et al (Ann Surg 94; 413-428 1981).

The ability of cells to anchor to a particular carrier is dependent on the properties of the carrier, the culturing conditions e.g. temperature, and the components of the culture medium. Culturing is usually

carried out in hard plastic flasks made from a material which is substantially inert to the growth medium and is non-cytotoxic to the cells. Polystyrene is a commonly used material for culture flasks.

One of the problems in using hard plastic flasks for the culture of epithelial cells for use as skin grafts is that the sheet of cells normally has to reach confluence before they can be harvested. The time taken to reach confluence may be long. Furthermore the layer of cells is not very strong mechanically and can easily be damaged when the unsupported cells are dislodged e.g. by using the enzyme dispase and handled unsupported.

International Patent Application No. WO89/03228 discloses a synthetic surgical dressing coated with collagen having human epidermal cells attached thereto. The method of producing the composite involves incubating the synthetic surgical dressing in a solution of collagen and evaporating the water to give a collagen coating on the surface of the synthetic dressing. The evaporation step is preferably conducted under sterile conditions to impede bacterial contamination.

It is known that the sheet of cells can be supported after it has been dislodged from the surface in order to facilitate handling and transfer to the wound surface. However this technique does not overcome the problems associated with hard surface cultures or the risks associated with dislodgement of the cells.

It is an object of the present invention, to overcome the above problems, by growing cells on a readily manageable, conformable carrier comprising a

hydrophilic, non-inhibitory to cell growth and non-cytotoxic polymer. The polymer of the present invention enables the transfer of a cell layer to the site of treatment before it has reached confluence.

According to the present invention a wound dressing comprises a conformable carrier having a wound-facing surface to which a layer of cultured mammalian cells is anchored, the carrier comprising a synthetic polymer layer which has a water uptake of at least 16w/w % and is non-cytotoxic and non-inhibitory to cell growth.

The present invention also provides a system for producing a wound dressing comprising a conformable carrier having a wound-facing surface to which a layer of cultured mammalian cells is anchored, the carrier being a synthetic polymer which is non-inhibitory to cell growth and non-cytotoxic and having a water uptake of at least 16 w/w %, together with a means for maintaining a aqueous culture medium containing said cells in contact with the wound facing surface of the carrier, the carrier being maintained in a submerged position in the culture medium.

The present invention further provides a method of wound treatment comprising the step of applying a wound dressing comprising a conformable carrier having a wound-facing surface to which a layer of cultured mammalian cells is anchored, the carrier being a synthetic polymer layer which is non-inhibitory to cell growth and non-cytotoxic and having a water uptake of at least 16 w/w %, to the area to be treated.

In further embodiment of the present invention there is provided a kit comprising a conformable

carrier having a wound facing surface, the carrier being a synthetic ploymer layer, which is non-inhibitory to cell growth and non-cytotoxic and having a water uptake of at least 16 w/w %, a container comprising a nutrient solution or growing mammalian cells and a vessel for containing the carrier and the nutrient solution.

The kit may additionally comprise a sample of mammalian cells.

The mammalian cells employed in the present invention are anchorage-dependent i.e. they require a surface on to which they can bind before they are able to proliferate.

By the term `conformable' we mean that the dressing will conform to changes in contours of the body portion to which the dressing is applied.

Preferably the synthetic polymer layer is a synthetic polymer film.

In one form, the carrier comprises a single layer of polymer. In another form the carrier comprises a laminate comprising two synthetic polymer layers, at least one polymer of said at least two layers being non-inhibitory to cell growth, non-cytotoxic and having a water uptake of at least 16 w/w%. The use of a laminate may be beneficial if the hydrophilic polymer swells or otherwise becomes fragile or difficult to handle after exposure to culture medium.

Where the wound dressing comprises a polymer layer having a water uptake of less then 16 w/w %, the cell layer may be anchored to this polymer layer.

Alternatively the cell layer may be anchored to the polymer layer having a water uptake of at least 16%.

In order for the carrier to have desirable surface properties which allows the anchorage of the cells, it may be surface treated. A suitable form of surface treatment is corona-discharge treatment. Corona discharge treatment increases the surface energy of a material and may provide improved conditions for cell anchorage. The effect of corona-discharge treatment can be assessed by measuring the contact angle of water on the treated material. A method for measuring contact angle will be described hereinafter. Aptly the contact angle should be reduced by at least 10%, favourably by at least 15% and preferably by at least 20%. Other suitable treatments which can be applied to the carrier include glow discharge or plasma treatment, chemical etching and flame treatment.

Thus in one embodiment of the present invention the wound dressing comprises a conformable carrier the carrier being a laminate of a polymer layer having a water uptake of at least 16 w/w % and a hydrophobic polymer layer, the polymers being non-inhibitory to cell growth and non-cytotoxic.

By hydrophobic polymer we mean that the polymer has a water uptake less than 16 w/w %.

Percentage water uptake of the polymer layer is assessed by the following method. A sample of the polymer layer is weighed in its dry state. This is then immersed in excess distilled water and left for a period of 24 hours at 20°C. The hydrated sample is then removed, excess water is removed from the surface of the sample and the sample re-weighed. The percentage increase in weight obtained, is the

percentage water uptake w/w%. The following equation may be used to calculate the percent water uptake (w/w %).

$$\text{w/w\% water uptake} = \frac{(\text{mass of sample + water}) - \text{mass of sample}) \times 100}{\text{mass of sample}}$$

The percentage water uptake w/w % of the carrier may be assessed by the above described method by taking a sample of the carrier rather than a sample of the polymer layer.

Preferably the carrier is transparent thus allowing the cells on the surface to be examined by a microscopy. However it is not essential that the cells be viewed during the period of cell culture and therefore non-transparent or opaque carriers may also be used in the dressing of the present invention.

The hydrophilic polymer may be a hydrogel or a hydrocolloid. Hydrogels are preferred as they are transparent.

The carrier should not be inhibitory to cell growth. A measure of such inhibition can be expressed as a percentage cell growth reduction as measured against cells allowed to grow in the absence of a test carrier as hereinafter described. Aptly the carrier should not result in more than 50% reduction in cell growth. More aptly it should not result in more than a 40% reduction in cell growth. Favourably it should not result in more than a 30% reduction in cell growth and preferably not result in more than a 20% reduction in cell gr wth.
The carrier should be non-cytotoxic. Cytotoxicity can be measured against a non-cytotoxic control material by a method as hereinafter described. Aptly

the cytoxicity of the carrier will not exceed 30%. More aptly the cytotoxicity of the carrier will not exceed 20%. Preferably the cytotoxicity of the carrier will not exceed 15%.

The carrier may be a laminate of at least two films or at least two sheets or a laminate comprising a film and a sheet.

Suitable synthetic polymers include hydrophilic polyurethane, polyetherpolyester (also known as a thermoplastic ether ester elastomer or a copolyester elastomer) for e.g. HYTREL (Trade Mark), polyetherpolyamide, e.g. PEBAX (Trade Mark), polyacrylamides and polyethylene oxide. Thus an apt laminate may comprise a polyurethane film coated with a material which allows attachment of anchorage dependent mammalian cells such as e.g. ethylene vinyl acetate.

The carrier may comprise a hydrogel, a hydrocolloid or other suitable polymer. Examples of suitable hydrogels include polyhydroxyethylmethacrylic acid (poly HEMA), cross-linked polyvinylacrylic acid (PVA), polyacrylic acid cross-linked with triallylsucrose (carbopol) and polyvinylpyrrolidone. Examples of suitable hydrocolloid materials include carboxymethylcellulose eg sodium carboxymethylcellulose. The carboxyurethylcellulose may be cross-linked or non cross-linked. The hydrocolloid may further comprise a polyisobutylene which serves to hold the hydrocolloid together. The hydrogel may be in the form of a film or a sheet. The sheet may be self-supporting. Alternatively th sheet may be supported by f r example a suitable support layer e.g. a polymeric film or polym r net. Where the she t is supported by a support layer the sheet and support

layer comprise the carrier.

Where the carrier comprises a hydrophobic polymer layer it may desirably be apertured to enable it to handle any exudate from the site of application particularly in the case of highly extruding wounds. Thus a continous polymer layer may be adapted to be perforated after the cell layer has reached the desired degree of confluency. Examples of such films, are the biaxially oriented films disclosed in for example in EP-0141592, GB-1055963 and GB-914489.

Aperturing the carrier as above described ensures that there is no build up of exudate in e.g. highly exuding wounds, since exudate can readily escape through the apertures and therefore does not build up under the dressings.

Perforation of the carrier may be carried out either before or after attachment of the cell layer using any suitable method such as hot pin perforation or slitting. Perforation after cell growth should be carried out with minimum cell disruption and loss. Perforation should be such as to provide an adequate open area for escape of wound exudate. The minimum open area will depend on the moisture vapour permeability of the film or the laminate.

Where the carrier is a laminate of two polymer films or nets in order that it should have the required flexibility and conformability, it should suitably have a thickness not exceeding 0.075mm. More suitably it should not have a thickness exceeding 0.05mm, and favourably not exceeding 0.04mm. More favourably the film will have a thickness between 0.005 and 0.03mm and preferably between 0.01 and 0.025mm for example,

0.015mm or 0.020mm. Where the carrier comprises a sheet and polymeric film or net it may have a thickness of the range of from 0.075mm up to about 5mm, the polymeric film or net having the above defined dimensions.

Meshing is conventionally carried out before grafting of a skin graft, to increase the surface area of the graft. In the same way, where the wound dressing of the present invention consists of polymeric films it may be conveyed through a conventional mesh - grafting device.

Films suitable for serving as the carrier may be flat or contoured. The contours may be produced for example by embossing. Suitably contoured films may also have apertures. Such films are described in W090/00398.

Aptly the carrier is permeable to moisture vapour, oxygen and carbon dioxide. In this way a dressing when in place on the wound will provide moist conditions allowing for the cells to remain viable while the wound heals. The carrier, whilst desirably being impervious to liquid water should have an upright moisture vapour transmission rate (MVTR) of at least $300gm^{-2}$ 24 hrs^{-1}. Aptly the carrier should have an upright MVTR of less than 2000 gm^{-2} 24 hrs^{-1}. The carrier should have an inverted MVTR of at least 2500 gm^{-2} 24 hrs^{-1}. Suitably the inverted MVTR should not exceed 25000 gm^{-2} 24 hrs^{-1}. A method for determining the upright and inverted MVTR of a substrate is given as follows:

The moisture vapour transmission rate (MVTR) may be measured by the Payne Cup method. This method uses a cup 1.5cm deep which has a flanged top. The inner

diameter of the flange provides an area of 10cm^2 of test material through which moisture vapour may pass. In this method 10ml of distilled water is added to the cup and a sample of the test material, large enough to completely cover the flange, is clamped over the cup. When the test material has an adhesive surface it is clamped with the adhesive surface facing into the cup. The complete assembly is then weighed and placed in a fan assisted electric oven where the temperature and relative humidity are maintained at 37°C and 10% respectively. The relative humidity within the oven is maintained at 10% by placing 1kg of anhydrous 3-8 mesh calcium chloride on the floor of the oven. After a suitable period of time, for example 17 hours, the cup is removed from the oven and allowed to cool for 20 minutes to reach room temperature. Ater reweighing the mass of water lost by vapour transmission is calculated. The moisture vapour permeability (MVP) is expressed in units of gm^{-2} 24 hrs^{-1} at 37°C, 100% to 10% relative humidity difference. This is the MVP when the test material is in contact with moisture vapour. To calculate the MVTR, the reading is corrected for standard thickness of test material i.e. a thickness of 1 thou. The MVP when the material is in contact with water may be measured using the same apparatus and making the necessary adjustments for thickness of test material, by simply placing the Payne cup in an inverted position in the oven so that liquid water (and not moisture vapour) is in contact with the test material.

In a modification of the invention the carrier may be formed from knitted, woven or non-woven synthetic polymers to f rm a tight web of small mesh size. After cell culture the web may be stretched to form a web having a larger mesh size without loss of

cells.

It is preferred to use autologous cultivated epithelial cells since these have little or no immunological rejection problems when applied to the host (patient). However it may also be possible to use non-autologous cells e.g. to produce an allograft or xenograft. Preferably the cells are keratinocytes. We have found that it is desirable not to allow the cell layer to reach confluence before transferring the wound dressing onto the wound site, thus enabling a suitable wound dressing to be produced within a few hours. It will be understood that as the cell layer is sub-confluent it will comprise a monolayer of cells.

The carrier may be sterilised by any suitable known methods of sterilisation. Suitable forms of sterilisation include ethylene oxide (allowing the required time for de-gassing) gamma-irradiation or steam sterilisation. The carrier should be washed after sterilisation, to remove any low molecular weight contaminants, for example where the carrier is a synthetic polymer to remove any unpolymerised monomer as such monomers can be cytotoxic. The washing process may comprise several sequential washes using sterile de-ionised water in sequential steps.

Alternatively the carrier may be aseptically produced.

In the system of the invention the carrier on which the cells are grown should preferably be easily rem vable from the other parts of the system, namely the means for maintaining the aqueous culture medium containing said cells in contact with the wound facing surface of the film. For example, where the carrier

forms a wall of the v ssel containing the culture medium, it should be readily removable from the other parts of the vessel. The carrier may form a part of all of the container in which the cell culture is grown. The other parts of the container or culture vessel may be formed from suitable materials conventionally used for the manufacture of tissue culture vessels. High impact polystyrene is preferred.

In an alternative embodiment the carrier may be laid down within a flask of an appropriate design adapted to allow the removal of the substrate. Where the carrier is an integral part of the culture flask, it may be removably sealed to the other parts of the flask for example by heat sealing or by means of an ahdesive. Preferably the carrier will form a flat surface and will aptly form the base of the flask.

Where the carrier is to be contained within the flask, the flask will be provided with a closeable opening having dimensions sufficient to enable the carrier to be readily removed without disruption of the cells anchored thereto.

Where apertured carrier are used to form an integral part of the culture flask these may be overlaid by e.g. a continuous film to keep the flask water tight and to maintain sterility. If the carrier is laid down within the flask it may be retained by, for example, a pre-sterilised stainless steel ring or, alternatively, by coating the carrier on one side with a layer of non-cytotoxic adhesive which is capable of maintaining tack in the presence of tissue culture medium.

Nutrients, growth factors or medicaments such as

antibiotics or antiflammatories may be incorporated into the aqueous medium in which the cell culture is grown. The nature and weight and/or volume of such additional ingredients are conventionally well known.

The wound dressing of this invention may be used for a variety of wounds. The dressing of the invention is particularly suitable for treating partial-thickness wounds that is those where only the epidermis and possibly part of the dermis is lost. Such wounds include for example skin graft donor sites, first or possibly second degree burns, shallow leg ulcers or pressure sores. The dressing is also suitable for treating full thickness wounds eg. venous ulcers. Continuous polymeric film carriers aptly act as barriers to bacteria whilst being sufficiently permeable to moisture vapour, oxygen and carbon dioxide to allow wound healing to occur at a desirable rate. If the substrate is perforated, a secondary dressing could be applied to maintain the desired degrees of moisture vapour, oxygen and carbon dioxide permeabilities. A suitable material is a polyurethane film dressing such as OPSITE (Trade Mark) which may be placed over the wound to create the same conditions. The dressing can suitably be left in place on the wound for a suitable period to allow the wound to become from 30-90% re-epithelialised, (healed) depending on the nature of the particular wound and the condition of the patient. At this time the dressing can be removed and replaced with a conventional wound dressing.

In 1975 Green et al proposed the use of transformed cell line 3T3 cells derived from the mouse as a feeder layer system in order to expand skin cultures. 3T3 cells synthesize factors which are ess ntial for the growth of keratinocytes which are

seeded at very low densitites. Furthermore 3T3 cells inhibit unwanted fibroblast growth. Skin cell preparations containing 3T3 cells first have to be g-irradiated (typically about 6000 rads) to inhibit cell division yet not kill the cells. Such irradiated cells survive for several days and during that time will synthesize and supply materials for the host keratinocyte cells. Eventually the 3T3 cells are expelled from the skin cell layer. However inevitably some mouse cells remain and thus will be grafted with the host keratinocytes.

In the dressings of the present invention the feeder cells such as 3T3 may be seeded into a medium in contact with the reverse side of the carrier. These cells will then synthesize materials for the host cells. Since the feeder cells do not come into contact with the host cells there is no need to irradiate them. When the carrier is removed from the culture flask, the reverse side may be washed to free floating feeder cells.

Where a feeder cell layer is employed with an apertured carrier, the apertures should be large enough to allow free exchange of the culture medium, but not large enough to allow cells to pass through. Aptly the aperture size should not be larger than 5μ across its largest dimensions. Suitably the aperture size will be from 0.5 to 2μ.

The dressings of the present invention may be prepared by suitably qualified personnel at the location where they are to be applied to the host. Thus suitably the dressing may be prepared according to the above disclosed method by a cell-culturist in a hospital cell-culture laboratory. Alternatively the

dressing may be prepared at a location remot from the location e.g. hospital where it is to be applied. In the latter case, the dressing may be transported under suitable conditions e.g. under a precisely controlled temperature. Thus the dressing may be cryopreserved ie. maintained at - 190°C and thereafter brought back to room temperature just prior to use. Alternatively the dressing may be transported in a ready-to-use state. Thus the dressing may be transported in a suitable incubator set at the desired temperature.

Contact angles are measured by the Wilhelmy Plate dynamic contact angle measurement system using a Cahn DCA-322 Dynamic Contact Angle Analyser. Both advancing and receding contact angles may be measured. The test liquid used may be water (HPLC grade). An immersion/withdrawal speed of 150 micron/sec may be used. Samples of films may be prepared by sticking the film onto a glass coverslip approx. 24mm by 30mm or by dip coating a slide from solution. The testing may be carried out at 23 deg. C/50% RH.

A suitable method for measuring cell growth reduction is the method disclosed in WO 91/13638.

The method of measuring percentage cellular cytotoxicity of the substrates is as disclosed in WO 91/13638.

Embodiments of the dressing systems of the invention will be illustrated by reference to the accompanying drawings. Referring to Figure 1, one wall the base, of a culture flask 1 comprises a laminate of an apertured film 2 and a c ntinuous film 3. The edges of the laminate are removably bonded to the other wall portions 5 of the flask 3. Culture media 4 and donor

cells can be introduced into flask 3 through neck 6 and sealed therein by stopper 7.

After the system has been incubated under suitable cell culture conditions, the laminate 2, 3 may be removed by peeling from flask 1.

Figure 2 illustrates a structure2, 3 formed by casting a first film 2 over a second film 3, film 3 having a plurality of raised portions 8. The resulting film 2, has a plurality of thin 9 and thickened 10 areas. On separation of film 2 from film 3, the thin areas 9 rupture to form apertures 12 as shown in Figure 3. The resulting apertured from which may subsequently be laminated to a polymer layer to form a laminate, suitable for use as a carrier in the dressing of the present invention.

Referring to Figure 3, the culture flask 1 is divided into compartments 21, 31 by a carrier comprising a hydrophilic polymer layer 14 and a perforated hydrophobic film 2.

Culture medium 4 is introduced into the flask and occupies both compartments 21, 31. Skin cells are seeded into compartment 21 through neck 6 whilst feeder cells such as 3T3 cells are seeded into compartment 31 through access port 11.

Nutrients, growth factors or medicaments such as anitbiotics or antiinflammatories may be incorporated into the aqueous medium in which the cell culture is grown. The nature of weight and/or volume of such additional ingredients are conventionally well known.

After the layer of skin cells has reached the required degree of confluence, the carrier is detached

from the wall portion 5 of the flask and removed from the access port 13.

The cell layer is preferably sub-confluent. Alternatively it may be confluent.

The invention may be illustrated by using any of the polymers given in the following table.

Product	Polymer	Water Uptake w/w %
Aquasorb	(polyvinylpyrrolidone)	430
Comfeel Ulcus	(carboxymethylcellulose)	60
Elastogel	(polyacrylamide)	250
Geliperm	(polyacrylamide/agar)	150
Granuflex E	(cmc)	25
HPU 45	(polyurethane)	70
HPU 75	(polyurethane)	460
Nu-gel	(pvp)	290
Cutinova Hydro	(polyurethane)	240

Examples

Human epithelial cells (keratinocytes) were grown on films and laminates comprising a polymer having a water uptake of greater than 16%. The materials and method used are detailed below.

The cells used were from the SCaBER cell line, supplied by American Type Culture Collection of Maryland USA.

The cells were labelled with a dye marker in order to facilitate their detection by optical microscopy. This was necessary because the cells were difficult to detect against the non-homogenous surfaces of some samples. The cells were labelled using a Sigma Immuno Chemicals PKH26 red fluorescent general cell linker kit supplied by Sigma Chemical Company of St Louis USA, according to the instructions suplied with the kit. The cells were then washed with serum-free culture medium before being re-suspended in fresh medium for culture on the samples.

Control experiments were performed in order to determine whether the fluorescent dye marker was taken up by the polymer substrates. The results showed that the dye was not absorbed by the polymers and was specific to the cells, so fluorescence observed on the experimental samples could be attributed to cells attached to the polymer surfaces.

The culture medium used was Earle's Minimum Essential Medium (MEM) + 10% foetal calf serum made up as follows:-

348 ml Earle's MEM (without L - glutamine), supplied by Gibco BRL.

40 ml	heat-inactivated foetal calf serum
4 ml	penicillin/streptomycin (5000IU/ml-5000 μg/ml), supplied by Gibco BRL.
4 ml	non-essential amino acids
4 ml	L-glutamine (100x) 200 mM, supplied by Gibco BRL.

The cells were seeded at a density of 10^6 cells per 3ml of medium.

The culture medium containing cells at the above concentration was placed in a petri-dish in contact with the sample film being tested and incubated at 37°C for 24 hours initially and then for further periods of 24 hours up to 3 days. The polymer substrate was then removed from the petri dish, carefully washed and then examined for the presence of attached cells under ultra-violet light using a NIKON inverted microscope fitted with a rhodium filter to detect the labelled red-fluorescing cells.

The polymer layers used were all thin films and were laid on the bottom of the petri dishes using metal rings to keep the film flat and make subsequent handling easier.

Polymer layers used for carrier were samples of hydrophilic films which are used in wound dressing constructions and so are known to be suitable for application to skin for medical purposes.

Example 1

Thermoplastic polyether-polyurethane extruded to form a film approximately 20μm thick, having a water uptake of 64% as measured by the method described

above, was used as the carrier layer. The film was laid on the base of a petri dish and culture medium containing cells was introduced onto the upper surface of the film. After incubation for 24 hours the carrier was removed and washed. Observation under u.v. light showed that cells had attached to the film surface. The film had swollen by absorbtion of culture medium.

Example 2

A film of thermoplastic polyether - polyurethane 20μm thick was laminated to a 50μm film of ethylene-vinyl acetate (EVA) to form a carrier layer. The EVA material had a water uptake of about 0.3%. The culture medium and cells were placed on the polyether

polyurethane side of the laminate. Some cells were observed to have attached after 24 hours, with increasing numbers of cells attaching after 2 and 3 days.

The sample appeared to have remained generally flat and ridges were seen in the surface of the film, due probably to the swelling of the hydrophilic film in the culture medium.

Example 3

The laminate described in Example 2 was used as the carrier layer and the cells were grown on the EVA side of the laminate. Cells were observed to have attached and grown on the film after 24 hours.

Example 4

The carrier used was a commercial dressing sold under the trademark "OPSITE IV3000" by Smith & Nephew Healthcare Ltd, which comprises a backing layer of a hydrophilic polyurethane film and a layer of acrylic pressure-sensitive adhesive attached to the wound-facing side thereof. The water-uptake of the dressing was measured as approximately 68%.

Cells were observed to colonise the non-adhesive, polyurethane side of the dressing.

Example 5

The carrier was the dressing used in example 4 with the adhesive side of the dressing used as the cell-carrying layer. Cells were observed to attach and proliferate on the adhesive.

Example 6

The carrier used was a laminate of two 20 micrometre thermoplastic polyether-polyurethane films as used in Example 1. As expected, cells were observed to be well dispersed over the film area in contact with the medium and attached to the film surface. The sample had a bubbled appearance after contact with the medium but this did not appear to unduly affect the attachment of cells to the surface.

CLAIMS

1. A wound dressing comprising a conformable carrier having a wound facing surface to which a layer of cultured mammalian cells is anchored, the carrier comprising a synthetic polymer layer which has a water-uptake of at least 16 w/w % and is non-cytotoxic and non-inhibitory to cell growth.

2. A wound dressing as claimed in claim 1, wherein said polymer layer is a synthetic polymer film.

3. A wound dressing as claimed in claim 1, wherein said polymer layer is a knitted, woven or non-woven fabric.

4. A wound dressing as claimed in any of claims 1 - 3, wherein said carrier comprises a single layer of a synthetic polymer which has a water uptake of at least 16% w/w.

5. A wound dressing as claimed in any of claims 1 - 3, wherein said carrier comprises a laminate comprising two synthetic polymer layers, at least one polymer of said at least two layers being non-inhibitory to cell growth, non-cytotoxic and having a water uptake of at least 16 w/w%.

6. A wound dressing as claimed in claim 5 comprising a conformable carri r which is a laminate of a polymer layer having a water-uptake of at least 16% w/w and a polymer layer having a water-uptake of less than 16w/w%, the polymers being non-inhibitory to cell growth and non-cytotoxic.

7. A wound dressing as claimed in claim 6, wherein the cells are anchored to said polymer layer having a water-uptake of at least 16 w/w%.

8. A wound dressing as claimed in claim 6, wherein the cells are anchored to said polymer layer having a water-uptake of less than 16%w/w.

9. A wound dressing as claimed in any preceding claim, wherein said wound-facing surface of said carrier has been surface-treated.

10. A wound dressing as claimed in claim 9, wherein said surface treatment has reduced the contact angle of said surface by at least 10%.

11. A wound dressing as claimed in any preceding claim, wherein said polymer layer comprises a film of a hydrophilic polyurethane, polyether-polyester, polyether-polyamide, polyacrylamide or polyethylene oxide.

12. A wound dressing as claimed in any preceding claim, wherein said carrier comprises an apertured polymer layer.

13. A wound dressing as claimed in any preceding claim, wherein the cells are epithelial cells.

14. A wound dressing as claimed in any preceding claim, wherein the cell layer is a sub-confluent layer.

15. A wound dressing as claimed in any preceding claim, wherein the cell layer is from 40% - 70% confluent.

16. A wound dressing as claimed in any preceding claim, wherein the cell layer is a monolayer.

17. A system for producing a wound dressing comprising a conformable carrier having a wound facing surface to which a layer of cultured mammalian cells is anchored, the carrier comprising a synthetic polymer layer which has a water-uptake of at least 16 w/w % and is non-cytotoxic and non-inhibitory to cell growth, together with a means for maintaining an aqueous cuture medium containing said cells in contact with the wound-facing surface of the carrier, the carrier being maintained in a submerged position in the culture medium.

18. A system as claimed in claim 17 wherein said means comprises a cultur vessel and said carrier layer forms an integral part of said vessel.

19. A system as claim d in claim 17, wherein said means comprises a vessel in which said carrier layer is arranged and supported.

20. A system as claimed in any of claims 17 - 19, wherein said carrier is supported upon a further liquid-impervious layer which forms a part of said containing means.

21. A system as claimed in any of claims 17 - 20 wherein said carrier layer is supported on a further layer, said further layer having projections and said carrier layer having a plurality of thinner layers corresponding to the areas of projections of the further layer.

22. A method of treating a wound comprising the step of applying a wound dressing comprising a conformable carrier having a wound facing surface to which a layer of cultured mammalian cells is anchored, the carrier comprising a synthetic polymer layer which has a water-uptake of at least 16 w/w % and is non-cytotoxic and non-inhibitory to cell growth, to the area to be treated.

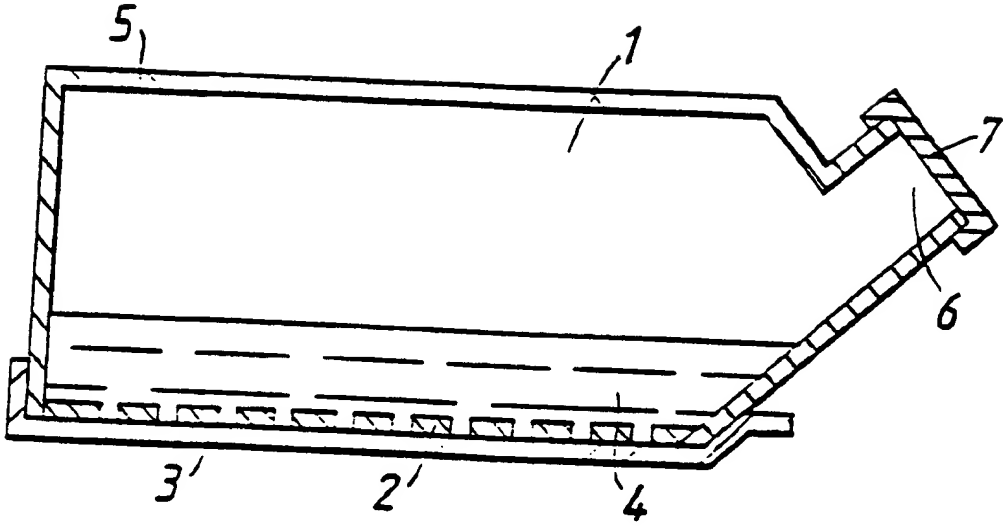
23. A kit comprising a conformable carrier having a wound facing surface, the carrier comprising a synthetic polymer layer which has a water-uptake of at least 16 w/w % and is non-cytotoxic and non-inhibitory to c ll growth, a contain r comprising a nutri nt solution for gr wing mammalian cells and a

vessel for containing the carrier and nutri nt solution.

24. A kit as claimed in claim 23 further comprising a sample of mammalian cells.

1/2

FIG. 1.



2/2

FIG. 2

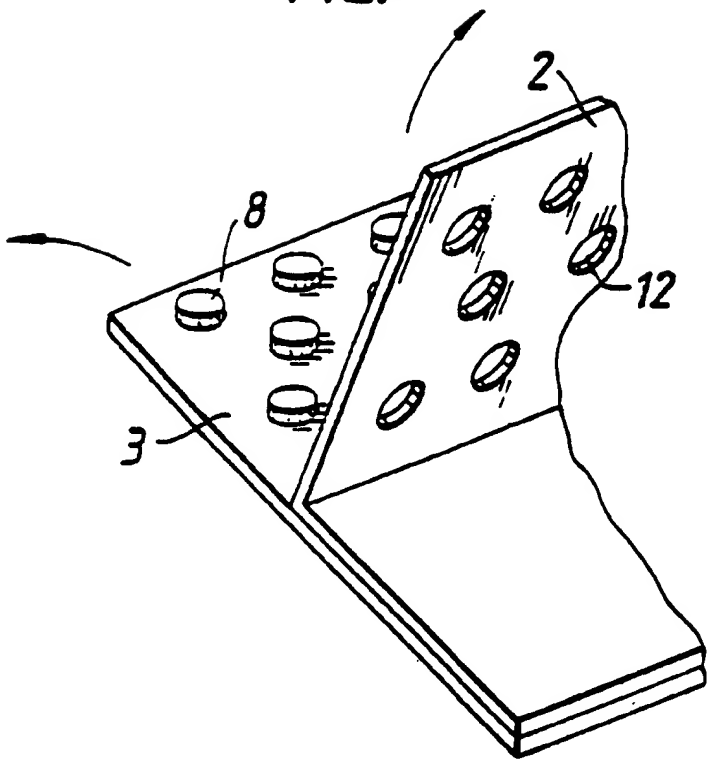
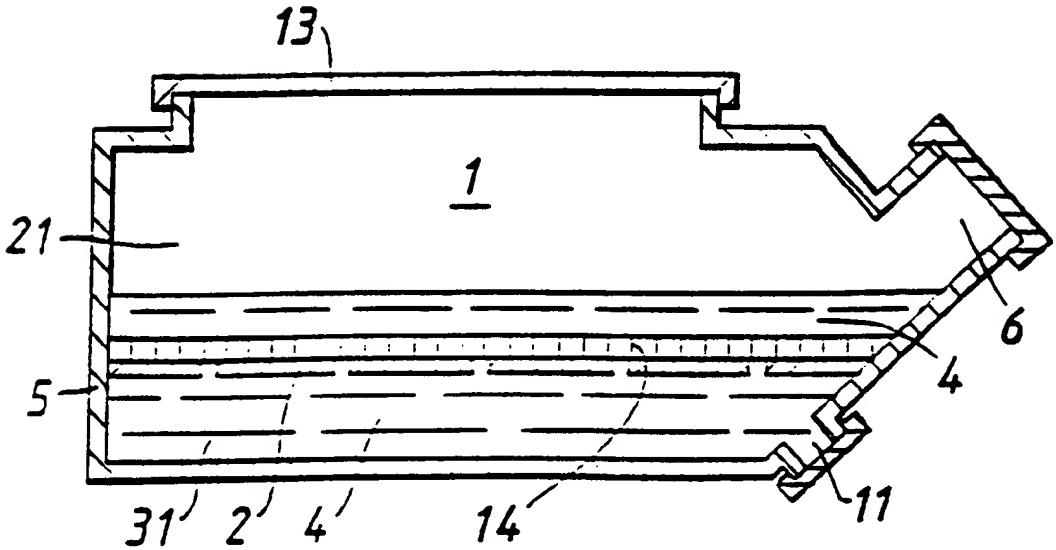


FIG. 3.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/01959

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L15/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 13638 (SMITH & NEPHEW) 19 September 1991 cited in the application see claims; figures	1-24
Y	WO,A,88 08448 (BAY MICHAEL) 3 November 1988 see claims	1-24
P,Y	WO,A,95 32743 (USTAV MAKROMOLEKULARNI CHEMIE ;LEKARSKA FAKULTA UNIVERZITY KA (CZ)) 7 December 1995 see claims; examples 1-4	1-24
A	WO,A,89 03228 (UNIV TEXAS) 20 April 1989 cited in the application see claims; examples	1-24

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 January 1997	04.02.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	ESPINOSA, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/01959

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,90 00595 (BANES ALBERT J) 25 January 1990 see examples I-V	1-24
A	EP,A,0 092 302 (BECTON DICKINSON CO) 26 October 1983 see claims	1-24

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 96/01959

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 22
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 22 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 96/01959

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9113638	19-09-91	AT-T-	139685	15-07-96
		AU-B-	649656	02-06-94
		AU-A-	7348591	10-10-91
		CA-A-	2065432	06-09-91
		DE-D-	69120527	01-08-96
		DE-T-	69120527	16-01-97
		EP-A-	0518920	23-12-92
		EP-A-	0681846	15-11-95
		ES-T-	2089195	01-10-96
		GB-A,B	2252562	12-08-92
		JP-T-	5504497	15-07-93
WO-A-8808448	03-11-88	AU-B-	614747	12-09-91
		AU-A-	1685288	02-12-88
		EP-A-	0355112	28-02-90
		JP-T-	2504221	06-12-90
WO-A-9532743	07-12-95	CZ-A-	9401314	17-04-96
WO-A-8903228	20-04-89	US-A-	5015584	14-05-91
		AU-A-	2625988	02-05-89
		US-A-	5334527	02-08-94
WO-A-9000595	25-01-90	US-A-	5122470	16-06-92
		AU-A-	3865489	05-02-90
		EP-A-	0435871	10-07-91
		JP-T-	4500455	30-01-92
EP-A-0092302	26-10-83	CA-A-	1201400	04-03-86
		DE-A-	3375157	11-02-88
		JP-C-	1732166	17-02-93
		JP-B-	4008033	13-02-92
		JP-A-	58201983	25-11-83